Transglutaminase-1 and Bathing Suit Ichthyosis: Molecular Analysis of Gene/Environment Interactions

Journal of Investigative Dermatology (2009) **129,** 2068–2071; doi:10.1038/jid.2009.18; published online 12 February 2009

TO THE EDITOR

Lamellar ichthyosis (LI) is a heterogeneous group of keratinization disorders characterized by scaling of the whole integument with differences in color and shape. Several LI cases (~40%) are due to mutations in the transglutaminase-1 (TGase-1) gene *TGM1* on chromosome 14q11 (Huber *et al.*, 1995; Russell *et al.*, 1995). TGase-1, which catalyzes the calcium-dependent cross-linking of proteins through the formation of ε-(γ-glutamyl)lysine isopeptide bonds, is synthesized in the upper differentiated epidermal layers, where it facilitates the formation of the cornified cell envelope by cross-linking structural proteins, such as loricrin and involucrin. Moreover, TGase-1 catalyzes the linkage of specialized ceramides to cell envelope proteins (Nemes *et al.*, 1999). Three clinical LI variants are related to mutations in *TGM1*. Patients with generalized LI are often born as collodion babies and develop a scaling on the entire body. A minority, referred as ''self-healing collodion baby,'' display a mild phenotype or heal spontaneously within the first few weeks of life (Reed *et al.*, 1972) and have particular *TGM1* mutations leading to impaired TGase-1 activity at higher intrauterine water pressure (Raghunath *et al.*, 2003).

Patients with bathing suit ichthyosis (BSI) develop a scaling pattern only affecting the trunk (Jacyk, 2005; Oji *et al.*, 2006; Arita *et al.*, 2007). We showed that the BSI phenotype is due to mutations in *TGM1* and hypothesized that the scaling pattern correlates with the local body temperature (Oji *et al.*, 2006). We show here that BSI mutations, when compared with wild type, (a) have a decreased enzyme activity (Figure 1a–b) and (b) exhibit a marked shift in temperature optimum from 37 to 31 °C (Figure 1c–e). Deficient activity of BSI mutants can be reconstituted by decreasing the temperature to below 33 °C (Table 1). These data explain the clinical phenotype with healthy areas on arms and legs having a lower body temperature.

Mutations of 10 patients (Oji *et al.*, 2006) have been studied regarding their impact on the enzymatic activity of TGase-1. Eleven mutations (Table 1) were expressed in HEK 293 cells, maintained at 32 °C. The TGase-1 cDNA was subcloned into the mammalian expression vector pcDNA3.1(+) (Invitrogen, Karlsruhe, Germany) and was used as a template for site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, Amsterdam, The Netherlands) following the manufacturer's instructions (http://www.stratagene.com). Eight of these mutations, namely Tyr276Asn, Arg126Cys, Arg264Trp, Arg307Gly, Arg264Gln, Arg687His, Arg315Cys, and Arg315His, are associated with BSI and three mutations (Arg142His, Arg389Pro, and Ser358fsX26) are further described in patients with classical LI. All constructs were confirmed by complete sequencing (Seqlab, Göttingen, Germany). At 96 hours after transfection, HEK293 cells were homogenized for 30 minutes on ice in lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 104 mM AEBSF, 0.08 mM aprotinin, 2 mM leupeptin, 4 mM bestatin, 1.5 mM pepstatin A, 1.4 mM E-64, pH 7.5). Cell lysates were assayed for protein using the BCA Protein Assay Kit (Pierce, Rockford, IL). Western blot analysis using a specific anti-TGase-1 antibody confirmed high expression levels of the wild type, and all but one of the mutants (Figure 1a). Several mutations — despite similar transfection efficiencies — showed reduced expression compared with the wild-type gene (Figure 1b). No expression was detectable in cell lysates that were repeatedly transfected with the mutant Ser358fsX26, indicating that this mutation causes protein instability. No TGase-1 expression was detected in HEK cells transfected with the pcDNA3.1 lacZ vector used as negative control and to determine transfection efficiency to show that different expression levels of the mutants are not because of different transfection efficiencies.

We analyzed the recombinantly expressed enzymes for activity between 25 and 45 °C by florescence spectrometry (LS55, Perkin Elmer, Rodgau, Germany; excitation: 332 nm, emission: 500 nm, slit: 5.0 nm). Each protein sample of 5 μg was incubated in prewarmed assay buffer (50 mM Tris-HCl, 10 mM $CaCl_2$, 10 mM reduced glutathione, 2.5% glycerol, 2.5% DMSO, 25 μM biotinylated cadaverine, and 20 μM *N,N*-dimethylcasein) and the activity was measured for 15 minutes. The incorporation of cadaverine into casein results in an increment of fluorimetric intensity and a shift in emission wavelength. The determined slope of an arithmetic mean of five measurements ($n=5$) served as a measure for TGase activity. Lysates of untransfected cells served as a negative control. The wild-type TGase-1 showed high activity over a wide temperature

Abbreviations: BSI, bathing suit ichthyosis; LI, lamellar ichthyosis; SHCB, self-healing collodion baby; TGase, transglutaminase

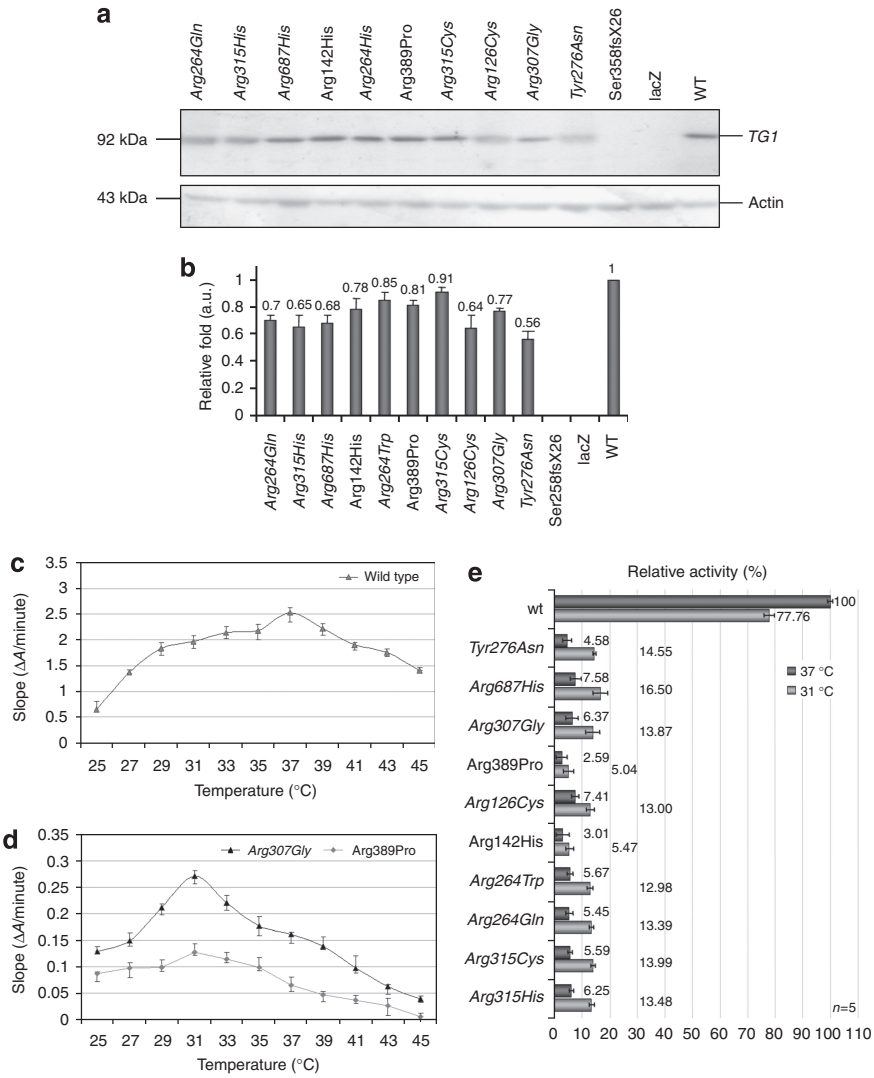

Figure 1. Expression (a and b) and activity (c–e) of recombinant transglutaminase-1 (TGase-1) mutants. HEK 293 cells were transiently transfected with 8 μg of pcDNA3.1(+) constructs containing wild-type or mutant TGase-1 or the β-galactosidase (lacZ) coding region using the Nanofectin transfection Kit according to the manufacturer's instructions (PAA, Cölbe, Germany). (**a**) Total protein of 15 μg was subjected to a discontinuous 10% SDS-PAGE under reducing conditions and afterward transferred onto a PVDF membrane (Immobilin P, Millipore, Eschborn, Germany). Western blot was developed using polyclonal antibodies raised against TGase-1 (1:2,000, N-Zyme BioTec, Darmstadt, Germany). Anti-actin antibodies were used as a control. (**b**) Protein levels were determined by densitometric quantification (ImageQuant, Molecular Dynamics, San Franciso, CA) and normalized to wild-type values, set as 1 in arbitrary units (a.u.). All transfections were performed in triplicate. (**c–e**) The activity of TGase-1 mutants and wild type were analyzed by fluorescence spectrometry. Total protein of 5 μg was incubated in pre-warmed assay buffer and the activity was measured for 15 minutes. TGase-1 activity is the slope of the arithmetic mean of five measurements ($n = 5$). Lysates of untransfected cells were used as a negative control. (**c**) Wild type; (**d**) examples for mutations described earlier in patients with lamellar ichthyosis type 1 (Arg389Pro) and mutations causing the BSI phenotype (Arg307Gly); (**e**) relative activity (%) of mutant TGase-1 proteins in comparison with the wild type at 37 and 31 °C. All BSI mutations show an activity above 10% at their temperature optimum at 31 °C and a dramatic decrease at 37 °C when compared with the wild type. The two mutations, Arg142His and Arg389Pro, described before in patients with generalized LI type 1 show a dramatic decrease of activity at both temperatures.

range with an optimum at 37 °C (Figure 1c). The mutations described for generalized LI display a strong decrease of activity (Figure 1d and e) without a clear optimum. All BSI mutations showed a decrease of activity and a shift of the temperature optimum from 37 to 31 °C. In all cases, the residual activity ranged between 13 and 16.5% at 31 °C, whereas the activity at 37 °C was reduced to <7.5% (Figure 1d and e).

Interestingly, seven of the eight BSI mutations are related to arginine substitutions. This residue is often involved in electrostatic interactions or hydrogen bonds and is referred to as easily mutated due to the deamination of 5′-CpG dinucleotides (Cooper and Youssoufian, 1988). Structural modeling based on the atomic structure of other TGases suggested that particular BSI mutations do not result in a major change of TGase-1 structure or protein folding (Oji *et al.*, 2006; Figure 2). We postulate that BSI mutations could influence hydrogen bonding, as several mutations (for example, Tyr276Asn, Arg264Trp, Arg264Gln, Arg307Gln, and

Table 1. Reconstitution of TGase-1 activity

Protein	Nucleotide change	Phenotype	Expression	Temperature (°C)		Relative activity (%)
				Pre-incubation	Assay	
Tyr276Asn	c.826T>A	BSI	+	40	31	13.57 ± 0.46
				30	31	14.01 ± 0.79
Arg126Cys	c.376C>T	BSI	+	40	31	13.61 ± 0.52
				30	31	12.99 ± 0.57
Arg264Trp	c.790C>T	BSI	+	40	31	12.98 ± 0.98
				30	31	11.34 ± 0.52
Arg307Gly	c.919C>G	BSI	+	40	31	13.87 ± 1.36
				30	31	12.89 ± 1.54
Arg264Gln	c.791G>A	BSI	+	40	31	13.99 ± 0.79
				30	31	13.44 ± 0.52
Arg687His	c.2060G>A	BSI	+	40	31	15.76 ± 0.33
				30	31	16.25 ± 1.13
Arg315Cys	c.843C>T	BSI	+	40	31	13.45 ± 0.52
				30	31	13.47 ± 0.25
Arg315His	c.844G>A	BSI	+	40	31	13.28 ± 0.34
				30	31	12.76 ± 0.56
Arg142His	c.425G>A	Classical LI	+	40	31	2.67 ± 0.89
				30	31	5.69 ± 0.65
Arg389Pro	c.1166G>C	Classical LI	+	40	31	2.75 ± 0.72
				30	31	4.88 ± 0.85
Ser358fsX26	c.1074delC	Classical LI	–	Not tested[1,2]		

BSI, bathing suit ichthyosisl; LI, lamellar ichthyosis; TGase, transglutaminase-1.
Data are displayed as relative activity in percentage (%).
[1]Nonsense mutation.
[2]After transfection, the mutant enzyme did not show any expression.

Arg687His) are involved in such interactions with surrounding residues. Removing or adding a polar group in the protein core leaves a hydrogen-bond donor or a acceptor unsatisfied (Boeshans *et al.*, 2007). Several residues affected in BSI (for example, Tyr276, Arg264, Arg307, and Arg315) are exposed to the solvent (Figure 2) and mutations could possibly influence the intrinsic salvation properties, local surface polarity, or surface hydrophobicity. Our current biochemical results on the reconstitution of deficient TGase-1 activity in BSI patients by decreasing the temperature to 31 °C further support these predictions from the atomic modeling. Samples pre-incubated at 30 and 40 °C for 30 minutes were assayed at 31 °C. The mutations associated with BSI display activities ranging from 11.34 to 16.25% (Table 1). In contrast to the temperature screening, no differences in activity could be detected. This is indicative of a reconstituted enzyme activity in the pre-incubated samples. In contrast, the classical mutations in TGase-1 revealed a decrease of activity after pre-incubation at 40 °C, similar to the activity at 37 °C.

As shown in Figure 1d and e and Table 1, at temperatures above 33 °C, activities of the BSI mutants decline to below 10% and clinical ichthyosis develops. The two mutations known to cause classical LI type 1 only show activities below 7.5%. This indicates the predisposition of relative activities below 10% to the development of the local scaling in BSI.

We conclude that the striking distribution of scaling in BSI is due to mutations that render TGase-1 sensitive to temperatures above 33 °C. A similar situation has been reported for autosomal-recessive oculocutaneous albinism, where hypopigmentation is related to local body temperature due to a temperature-sensitive mutation in human tyrosinase. It has been proposed that the thermo-sensitive tyrosinase directly interferes with thermal stability of the protein (Giebel *et al.*, 1990; Berson *et al.*, 2000). Another cutaneous phenotype in which body temperature plays an important role has been described for a mutation in *CDKN2A* (cyclin-dependent kinase inhibitor 2A), which is associated with an increased rate of nevus development and density on warmer body regions (Florell *et al.*, 2004). It is tempting to speculate that gene/environment interactions relating to body temperature may be relevant for further skin diseases and are not adequately appreciated so far.

CONFLICT OF INTEREST

The authors state no conflict of interest.

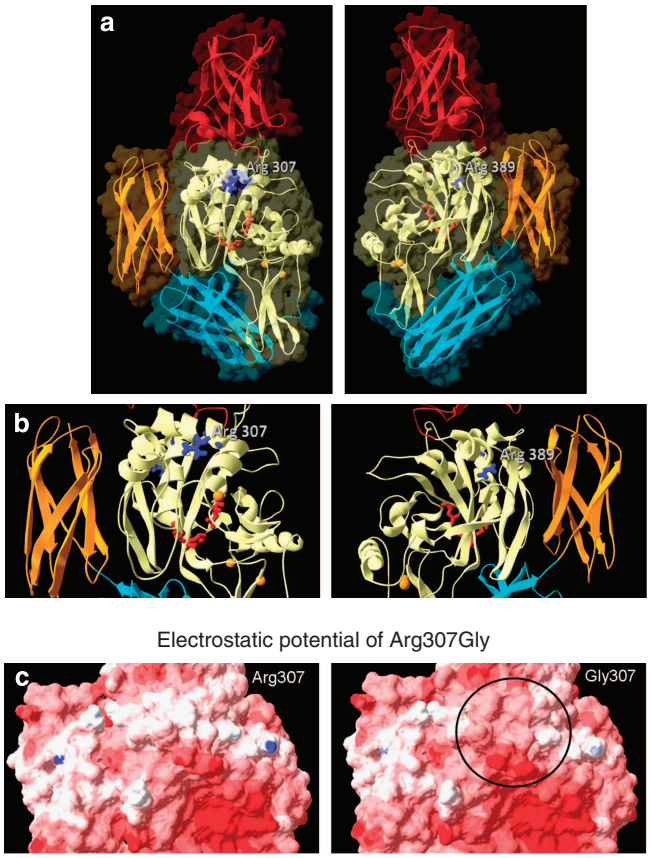

Figure 2. Comparative three-dimensional *in silico* modeling of the human TGase-1 structure concerning the mutations Arg307Gly (BSI) and Arg389Pro (classical LI type 1). After alignment, the molecular structure of human TGase-1 was predicted by Swiss Model (Kopp and Schwede, 2004). Modeling, energy minimization, and amino-acid substitutions were performed with DeepView SwissPDB Viewer 3.7 (Kaplan and Littlejohn, 2001) based on the structure of TGase-3 (Ahvazi *et al.*, 2003). (**a** and **b**) Ribbon image of the model of human TGase-1 structure with Arg307 in the left panel and Arg389 in the right panel (blue). The four domains are the β-sandwich (red), the catalytic core (yellow), the β-barrel 1 (blue), and β-barrel 2 (orange). The calcium ions are shown in orange. The side chains of the amino acids of the catalytic triad are drawn in ball-and-stick (red). (**a**) Overview of TGase-1 structure and (**b**) details of the ribbon image of TGase-1 show the location of the mutations Arg307 and Arg389. (**c**) View of the electrostatic surface potential of TGase-1 cavity surrounding residue Arg307 and Gly307. The electrostatic potentials have been mapped onto the surface plan from −15 kT (deep red) to +15 kT (deep blue). Arg307 is located in the core domain and hydrogen bonded to Tyr303 and Asn335. It is exposed to solvent and could possibly influence the intrinsic salvation properties of the protein. Arg389 is a highly conserved residue located in the center of the core domain of the TGase 1 peptide but buried within the molecule. An alteration of Arg389 could possibly result in an impairment of protein folding and therefore in a complete loss of activity. Arg389Pro shows no pronounced differences in electrostatic potential (data not shown).

ACKNOWLEDGMENTS

This work is supported by the Selbsthilfe Ichthyose e.V. and by the Bundesministerium für Bildung und Forschung as part of the Network for Rare Diseases NIRK (GFGM01143901).

Karin Aufenvenne[1], Vinzenz Oji[1,2], Tatjana Walker[1], Christoph Becker-Pauly[3], Hans Christian Hennies[4], Walter Stöcker[3] and Heiko Traupe[1]

[1]Department of Dermatology, University of Muenster, Münster, Germany; [2]University of Münster, Interdisciplinary Center of Clinical Research, Münster, Germany; [3]Institute of Zoology, Johannes Gutenberg University, Mainz, Germany and [4]Cologne Center for Genomics, Division of Dermatogenetics, University of Cologne, Cologne, Germany
E-mail: karin.aufenvenne@ukmuenster.de

REFERENCES

Ahvazi B, Boeshans KM, Idler W, Baxa U, Steinert PM (2003) Roles of calcium ions in the activation and activity of the transglutaminase 3 enzyme. *J Biol Chem* 278:23834–41

Arita K, Jacyk WK, Wessagowit V, van Rensburg EJ, Chaplin T, Mein CA *et al.* (2007) The South African ''bathing suit ichthyosis'' is a form of lamellar ichthyosis caused by a homozygous missense mutation, p.R315L, in transglutaminase 1. *J Invest Dermatol* 127:490–3

Berson JF, Frank DW, Calvo PA, Bieler BM, Marks MS (2000) A common temperature-sensitive allelic form of human tyrosinase is retained in the endoplasmic reticulum at the nonpermissive temperature. *J Biol Chem* 275:12281–9

Boeshans KM, Mueser TC, Ahvazi B (2007) A three-dimensional model of the human transglutaminase 1: insights into the understanding of lamellar ichthyosis. *J Mol Model* 13:233–46

Cooper DN, Youssoufian H (1988) The CpG dinucleotide and human genetic disease. *Hum Genet* 78:151–5

Florell SR, Meyer LJ, Boucher KM, Porter-Gill PA, Hart M, Erickson J *et al.* (2004) Longitudinal assessment of the nevus phenotype in a melanoma kindred. *J Invest Dermatol* 123:576–82

Giebel LB, Strunk KM, King RA, Hanifin JM, Spritz RA (1990) A frequent tyrosinase gene mutation in classic, tyrosinase-negative (type IA) oculocutaneous albinism. *Proc Natl Acad Sci USA* 87:3255–8

Huber M, Rettler I, Bernasconi K, Frenk E, Lavrijsen SP, Ponec M *et al.* (1995) Mutations of keratinocyte transglutaminase in lamellar ichthyosis. *Science* 267:525–8

Jacyk WK (2005) Bathing-suit ichthyosis. A peculiar phenotype of lamellar ichthyosis in South African blacks. *Eur J Dermatol* 15:433–6

Kaplan W, Littlejohn TG (2001) Swiss-PDB Viewer (deep view). *Brief Bioinfom* 2:195–7

Kopp J, Schwede T (2004) The SWEISS-MODEL repository of annotated three-dimensional protein structure homology models. *Nucleic Acids Res* 32:230–4

Nemes Z, Marekov LN, Fesus L, Steinert PM (1999) A novel function for transglutaminase 1: attachment of long-chain omega-hydroxyceramides to involucrin by ester bond formation. *Proc Natl Acad Sci USA* 96:8402–7

Oji V, Hautier JM, Ahvazi B, Hausser I, Aufenvenne K, Walker T *et al.* (2006) Bathing suit ichthyosis is caused by transglutaminase-1 deficiency: evidence for a temperature-sensitive phenotype. *Hum Mol Genet* 15:3083–97

Raghunath M, Hennies HC, Ahvazi B, Vogel M, Reis A, Steinert PM *et al.* (2003) Self-healing collodion baby: a dynamic phenotype explained by a particular transglutaminase-1 mutation. *J Invest Dermatol* 120:224–8

Reed WB, Herwick RP, Harville D, Porter PS, Conant M (1972) Lamellar ichthyosis of the newborn. A distinct clinical entity: its comparison to the other ichthyosiform erythrodermas. *Arch Dermatol* 105:394–9

Russell LJ, DiGiovanna JJ, Rogers GR, Steinert PM, Hashem N, Compton JG *et al.* (1995) Mutations in the gene for transglutaminase 1 in autosomal recessive lamellar ichthyosis. *Nat Genet* 9:279–83